AMENDMENTS TO THE SPECIFICATION

Please replace the section beginning on page 9 entitled "BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS as follows:

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

~~Figure 1. Intensity differentials using multiple different probes.~~

~~Figure 4. PCR products (approximately 0.1 ng/□l dye-labeled PCR product with and without 10 ng/□l unlabeled PCR product) were denatured at elevated pH for five minutes at room temperature, chilled on ice for five minutes, and adjusted to neutral pH to enable hybridization. Hybridized and re-annealed samples were diluted 1/1000 in 100 mM glycylglycine, 0.1 % hydroxymethylpropyl cellulose, pH 8.2, and 50 ul of sample was loaded into a coated 200 um glass capillary. Electrophoresis was for five minutes at 2000 volts (constant). Photon cross correlation data were obtained by excitation of the sample with 635 nm lasers at 1 mW power, and detection at appropriate wavelength to detect fluorescence from the dye. The current was approximately 80 □amperes. Approximately 1,000 crosscorrelation events were detected in each experiment. Crosscorrelation data represents crosscorrelation of photon bursts weighted by an intensity factor. Consequently brighter crosscorrelation for an equal number of crosscorrelation events will produce a peak with increased amplitude.~~

~~The labeled PCR product alone was detected at approximately 650 msec (elapsed time between detection at the two detector positions). In the presence of excess unlabeled PCR product the peak at 650 msec was present, but it was reduced in intensity (decreased peak amplitude) due to hybridization with its unlabeled complement (Figure 4a and 4b).~~

~~Figure 5. Photon bursts were analyzed using the instrument software to group those bursts generated by molecules. Figures 5a and 5b show a time course segment of the data for molecule photon bursts. Labeled-unlabeled hybrid molecules have photon bursts of 50 photons – photon bursts of 100 are seen with labeled-only molecules.~~

~~Figure 6. Annealed samples (see figures 4a and 4b) were diluted 1/10,000 in 100 mM glycylglycine, 0.1 % hydroxymethylpropyl cellulose, pH 8.2 and pumped into a coated capillary at 1 □l/minute for 3 – 5 minutes. Excitation was with 635 nm lasers at 1 mW, and detection was at the appropriate wavelength to detect fluorescence.~~

~~Photon bursts were analyzed using the instrument software to group photon bursts into those groups of photons originating from individual molecules. Looking at time plots of the molecule photon burst intensities (figure 5) we see that labeled-unlabeled hybrid molecules typically have~~

~~burst intensities of about 50 photons, while labeled-only molecules have photon bursts of about 100.~~

~~Histograms of number of molecules vs. photon intensity were plotted (figures 6a and 6b). With uniform illumination, identically-labeled individual molecules will produce equivalent photon bursts. The label-only molecules will be twice as bright (produce larger photon bursts) compared to the labeled-unlabeled hybrids. However, because the molecules are illuminated here by a non-uniform beam, the detectable photon bursts, when looked at in composite, have a distribution of intensities. Nonetheless, we can see from the molecule photon bursts (Figure 6) that labeled-unlabeled hybrid molecules (1 X Intensity) have photon bursts of about 50 photons, while the labeled-only molecules (2 X Intensity) have photon burst sizes of about 100 photons. Using a cut-off point of 50 photons we also can see the overall distribution of 2XI molecules in the histogram of molecule intensities and can determine the number of labeled-unlabeled hybrid molecules detected in the experiment..~~

Figure 1. Intensity differentials using multiple different probes.

Figure 2. Schematic diagram of the basic apparatus for single molecule detection using laser induced fluorescence.

Figure 3. Heart of the SMD Instrument showing glass capillary tube.

Figure 4. PCR products (approximately 0.1 ng/µl dye-labeled PCR product with and without 10 ng/µl unlabeled PCR product) were denatured at elevated pH for five minutes at room temperature, chilled on ice for five minutes, and adjusted to neutral pH to enable hybridization. Hybridized and re-annealed samples were diluted 1/1000 in 100 mM glycylglycine, 0.1 % hydroxymethylpropyl cellulose, pH 8.2, and 50 ul of sample was loaded into a coated 200 um glass capillary. Electrophoresis was for five minutes at 2000 volts (constant). Photon cross correlation data were obtained by excitation of the sample with 635 nm lasers at 1 mW power, and detection at appropriate wavelength to detect fluorescence from the dye. The current was approximately 80 amperes. Approximately 1,000 cross correlation events were detected in each experiment. Cross correlation data represents cross correlation of photon bursts weighted by an intensity factor. Consequently brighter cross correlation for an equal number of cross correlation events will produce a peak with increased amplitude.
The labeled PCR product alone was detected at approximately 650 msec (elapsed time between detection at the two detector positions). In the presence of excess unlabeled PCR product the peak at 650 msec was present, but it was reduced in intensity (decreased peak amplitude) due to hybridization with its unlabeled complement (Figure 4a and 4b).

Figure 5. Photon bursts were analyzed using the instrument software to group those bursts generated by molecules. Figures 5a and 5b show a time course segment of the data for molecule photon bursts. Labeled-unlabeled hybrid molecules have photon bursts of 50 photons – photon bursts of 100 are seen with labeled-only molecules.

Figure 6. Histogram of photon intensities of hybridized unitized probe. Annealed samples (see figures 4a and 4b) were diluted 1/10,000 in 100 mM glycylglycine, 0.1 % hydroxymethylpropyl cellulose, pH 8.2 and pumped into a coated capillary at 1 µl/minute for 3 – 5 minutes. Excitation was with 635 nm lasers at 1 mW, and detection was at the appropriate wavelength to detect fluorescence. Photon bursts were analyzed using the instrument software to group photon bursts into those groups of photons originating from individual molecules. Looking at time plots of the molecule photon burst intensities (figure 5) we see that labeled-unlabeled hybrid molecules typically have burst intensities of about 50 photons, while labeled-only molecules have photon bursts of about 100. Histograms of number of molecules vs. photon intensity were plotted (figures 6a and 6b). With uniform illumination, identically-labeled individual molecules will produce equivalent photon bursts. The label-only molecules will be twice as bright (produce larger photon bursts) compared to the labeled-unlabeled hybrids. However, because the molecules are illuminated here by a non-uniform beam, the detectable photon bursts, when looked at in composite, have a distribution of intensities. Nonetheless, we can see from the molecule photon bursts (Figure 6) that labeled-unlabeled hybrid molecules (1 X Intensity) have photon bursts of about 50 photons, while the labeled-only molecules (2 X Intensity) have photon burst sizes of about 100 photons. Using a cut-off point of 50 photons we also can see the overall distribution of 2XI molecules in the histogram of molecule intensities and can determine the number of labeled-unlabeled hybrid molecules detected in the experiment.

Figure 7. Example of output from existing laboratory device. The upper two traces show the number of photons as a function of time (each unit represents 2 ms) for the two channels. Each large spike represents a fluorescence detection event over the background levels. The bottom trace represents the cross-correlation of the events for channel one with the events for channel two over a 30 sec period with a single peak at 700 msec.

AMENDMENTS TO THE DRAWINGS

Please replace the seven (7) original figures with the attached replacement sheets 1/7 through 7/7.